UNIVERSITI PUTRA MALAYSIA

SECONDARY METABOLITES FROM STEM BARK OF GERONGGANG (CRATOXYLUM ARBORESCENS (VAHL) BL.) AND KETEMAU (CRATOXYLUM GLAUCUM K.) AND THEIR BIOLOGICAL ACTIVITIES

SIM WEI CHUNG

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By

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

SECONDARY METABOLITES FROM STEM BARK OF GERONGGANG (*CRATOXYLUM ARBORESCENS* (VAHL) BL.) AND KETEMAU (*CRATOXYLUM GLAUCUM* K.) AND THEIR BIOLOGICAL ACTIVITIES

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September 2010

Chairman : Prof. Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Phytochemical and biological studies were carried out on two species from the Guttiferae family, *Cratoxylum arborescens* and *Cratoxylum glaucum*. The chemical investigation discovered triterpenoids, quinones and xanthones. These compounds were isolated using common chromatographic techniques and were identified using modern spectroscopic techniques such as 1D and 2D NMR, MS, IR and UV.

Cratoxylum arborescens (stem bark) afforded friedelin, stigmasterol, fuscaxanthone C and two anthraquinone, vismiaquinone and 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone. Meanwhile, studies on *Cratoxylum glaucum* (stem bark)

revealed the presence of three xanthones, *α*-mangostin, *β*-mangostin and fuscaxanthone C, along with an anthraquinone, 1,8-dihydroxy-3-methoxy-6-methylanthraquinone, friedelin, *β*-sitosterol and betulinic acid. This is the first report of *α*-mangostin, *β*-mangostin and betulinic acid from *Cratoxylum glaucum*. The acetylation of fuscaxanthone C has successfully yielded dimethylmangostin acetate.

Antioxidant properties of the crude extracts of these two species were tested using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and compared to a well known antioxidant, ascorbic acid. The crude methanol extracts of both *Cratoxylum arborescens* and *C. glaucum* showed high antioxidant activities with low EC_{50} values of 10.40 and 7.48 ppm, respectively, and are comparable to that of ascorbic acid, 5.17 ppm. The crude chloroform extract of *C. arborescens* also gave good activity, with an EC_{50} value of 31.04 ppm. The total phenolic content of the methanol extracts of both species were measured using Folin-Ciocalteu method.

The hexane and ethyl acetate extracts of *Cratoxylum glaucum* and the hexane and chloroform extracts of *Cratoxylum arborescens* demonstrated low IC_{50} values against the MCF7 cancer cell line. Meanwhile, the ethyl acetate and methanol extracts of *Cratoxylum glaucum* and all the extracts of *Cratoxylum arborescens* were active against HL-60 cancer cell line.

The antibacterial activity test was also carried out using eight bacteria, namely, *Bacillus cereus*, *Micrococcus luteus,* Methicillin-Sensitive *Staphylococcus Aureus* (MSSA), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*. However, all the crude extracts gave only weak or no inhibition to these bacteria.

The larvicidal tests were performed against the larvae of *Aedes aegypti* using the WHO (1981) standard procedures with slight modifications. All the extracts of *Cratoxylum arborescens* and *C. glaucum* were found to be inactive against the larvae of *Aedes aegypti*.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

METABOLIT SEKUNDER DARIPADA KULIT GERONGGANG (*CRATOXYLUM ARBORESCENS* (VAHL) BL.) DAN KETEMAU (*CRATOXYLUM GLAUCUM* K.) DAN AKTIVITI BIOLOGI

Oleh

SIM WEI CHUNG

September 2010

Pengerusi : Prof. Gwendoline Ee Cheng Lian, PhD

Fakulti : Sains

Kajian fitokimia dan biologi telah dilakukan terhadap dua spesies daripada famili Guttiferae, iaitu *Cratoxylum arborescens* dan *Cratoxylum glaucum*. Penyelidikan kimia ini telah menemukan triterpenoid, kuinon dan xanton. Sebatian ini diasingkan dengan menggunakan teknik kromatografi biasa dan dikenal pasti dengan menggunakan teknik spektroskopi seperti 1D dan 2D NMR, MS, IR dan UV.

Daripada *Cratoxylum arborescens* (kulit batang), diperolehi friedelin, stigmasterol, fuskaxanton C dan dua antrakuinon, vismiakuinon dan 3-geraniloksi-6-metil-

1,8-dihidroksiantrakuinon. Manakala, kajian terhadap *Cratoxylum glaucum* (kulit batang) telah menemukan tiga xanton, *α*-mangostin, *β*-mangostin dan fuskaxanton C, bersama dengan satu antrakuinon, 1,8-dihidroksi-3-metoksi-6-metilantrakuinon, friedelin, *β*-sitosterol and asid betulinik. Ini merupakan laporan pertama bagi *α*-mangostin, *β*-mangostin dan asid betulinik daripada *Cratoxylum glaucum*. Asetilasi fuskaxanton C telah berjaya menghasilkan dimetilmangostin asetat.

Keupayaan antipengoksidaan ekstrak dua spesis ini telah diuji dengan menggunakan cara penghapusan radikal 2,2-difenil-1-pikrilhidrazil (DPPH) dan dibandingkan dengan agen antipengoksidaan yang umum, iaitu asid askorbik. Ekstrak metanol bagi kedua-dua *Cratoxylum arborescens* dan *C. glaucum* menunjukkan aktiviti anti-pengoksidaan yang tinggi dengan EC_{50} yang rendah, iaitu 10.40 dan 7.48 ppm, berbanding dengan asid askorbik, iaitu 5.17 ppm. Ekstrak kloroform *C. arborescens* juga menunjukkan aktiviti yang baik dengan nilai EC_{50} pada 31.04 ppm. Jumlah kandungan fenolik bagi ekstrak methanol bagi kedua-dua spesis telah disukat dengan menggunakkan kaedah Folin-Ciocalteu.

Ekstrak heksana dan etil asetat *Cratoxylum glaucum* dan ekstrak heksana dan kloroform *Cratoxylum arborescens* menunjukkan nilai IC_{50} yang rendah terhadap sel barah MCF7. Manakala, ekstrak etil asetat dan metanol daripada *Cratoxylum glaucum* dan semua ekstrak daripada *Cratoxylum arborescens* adalah aktif terhadap sel barah HL-60.

Ujian aktiviti antibakteria dikaji terhadap lapan jenis bakteria, iaitu *Bacillus cereus*, *Micrococcus luteus,* Methicillin-Sensitive *Staphylococcus Aureus* (MSSA), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* dan *Enterobacter aerogenes*. Akan tetapi, kesemua ekstrak tidak menunjukkan aktiviti ataupun hanya menunjukkan aktiviti lemah terhadap bakteria tersebut.

Ujian ke atas larva telah dilakukan dengan larva *Aedes aegypti* dengan mengikuti prosedur piawai WHO (1981) dengan sedikit pengubahsuaian. Kesemua ekstrak *Cratoxylum arborescens* dan *C. glaucum* didapati tidak aktif terhadap larva *Aedes aegypti*.

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I certify that a Thesis Examination Committee has met on 21 September 2010 to conduct the final examination of Sim Wei Chung on his thesis entitled "Secondary Metabolites from the Stem Bark of Geronggang (*Cratoxylum arborescens* (Vahl) Bl.) and Ketemau (*Cratoxylum glaucum* K.) and Their Biological Activities" in accordance with the Universities and University College Act 1971 and the Constitution of Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the candidate be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotation and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently submitted, for any degree at Universiti Putra Malaysia or other institutions.

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SIM WEI CHUNG

Date: 21 September 2010

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